Materials Science and Engineering C 58 (2016) 316–323

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Polyacrylic acids–bovine serum albumin complexation: Structure and dynamics

Mohamed Othman, Adel Aschi *, Abdelhafidh Gharbi

Labeoratoire de Physique de la Matière Molle et Modélisation Electromagnétique, Faculté des Sciences de Tunis, Campus Universitaire, 2092, Université de Tunis El Manar, Tunisia

ARTICLE INFO

Article history:
Received 12 March 2015
Received in revised form 23 August 2015
Accepted 25 August 2015
Available online 5 September 2015

Keywords:
Complex phase
Turbidity
Dynamic light scattering
Nuclear magnetic resonance
Electrostatic potential

ABSTRACT

The study of the mixture of BSA with polyacrylic acids at different masses versus pH allowed highlighting the existence of two regimes of weak and strong complexation. These complexes were studied in diluted regime concentration, by turbidimetry, dynamic light scattering (DLS), zeta-potential measurements and nuclear magnetic resonance (NMR). We have followed the pH effect on the structure and properties of the complex. This allowed refining the interpretation of the phase diagram and understanding the observed phenomena. The NMR measurements allowed probing the dynamics of the constituents versus the pH. The computational method was used to precisely determine the electrostatic potential of BSA and how the polyelectrolyte binds to it at different pH.

1. Introduction

The systems involving interactions between polyelectrolytes and oppositely charged spheres are present in many areas and fields of application [1–3]. These systems are found in nature, which gives them a biological interest [4]. One of the newest and most interesting applications is the transfer of genes associated with gene therapy. In this case, the association between the DNA of vectorized gene and a cationic polymer forms the complex. The polyelectrolytes can also stabilize mineral particles in solution or encapsulate them for delivery. These encapsulation capabilities are also useful in food processing; where charged particles are biological macromolecules, mainly proteins. The grafted polyelectrolytes on surfaces can also be used to trap particles on these surfaces [5–7].

In general, the complex formation is based on electrostatic interactions between opposite charges of polyelectrolyte and protein. Various physico-chemical parameters can influence the electrostatic interactions and the complex formation. To better understand these parameters, we try to study one system where we were able to control the parameters governing the complex formation, in particular, the electric charge. We choose macroscopic studies (phase diagram) to determine the structures formed at the scale of objects based on the physicochemical parameters that can modify the interactions in the system.

This study aimed at studying the effect of pH on the structure and properties of the mixture system composed of bovine serum albumin (BSA)–polyacrylic acid (PAA). The composed systems of the polyelectrolyte and proteins are very varied and their related current studies are very important [8–12]. In our case, the chosen protein is the bovine serum albumin (BSA) that has a globular shape in native state [13,14], stable in solution and does not form oligomers. Its isoelectric pH_i is equal to 4.9, which allows varying the total charge (positive/negative) on a reasonable range of pH. Finally, this protein is commercially available in large quantities and with sufficient purity. To overcome the problem of lack of system stability, making it difficult to perform experiments, we have chosen to use a polyelectrolyte; where we can control the parameters governing the formation of complexes in particular the electric charge of the chains. This is possible by using the polyelectrolyte whose charge density is pH dependent. The polyacid that we have chosen is polyacrylic acid (PAA). It is linear, flexible and has different lengths. In this article, we discuss the phase diagrams obtained at low concentration. The goal is to follow the changes in turbidity, zeta potential and the hydrodynamic radius of the complex versus pH. Still, this study was performed for three different masses of PAA ($M_w = 5.7$ k, 57 k and $M_w = 118$ k) to monitor the effect of pH on the chain length of the polyelectrolyte. We also examined the influence of the ratio r of constituent concentrations on the properties of complexes. The study of our systems by NMR will clarify the influence of the structure on the complex dynamics. Finally, we extended our work by studying the computational method of the electrostatic potential of BSA. The electrostatic potential helped us in the identification of functional sites at the surface of a protein and might be visualized by color-coding the molecular surface versus the potential values. The aim of this part of work is to

---

* Corresponding author at: Laboratoire de Physique de la Matière Molle et de la Modélisation Electromagnétique, Faculté des Sciences de Tunis, Campus Universitaire, 1060, Université de Tunis El-Manar, Tunisia.
E-mail address: aschi13@yahoo.fr (A. Aschi).

http://dx.doi.org/10.1016/j.msec.2015.08.057
0928-4931/

precisely know how the polyelectrolyte binds with the protein when they have the same sign charge.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA) (essential fatty acid free) is purchased from Sigma Chemical Co and is a large globular protein (molecular weight = 67 kDa and residues = 585) with a good essential amino acid profile.

The used protein concentration of complex for this study was 0.1% (*w*/*v*). The polyacid used in our experiments was polyacrylic acid (PAA) hydrogenated and deuterated. The molecular weight average weight M_w, the polydispersity I and the polymerization index N were presented in Table 1. Polyacrylic acids were obtained from Polysciences Inc. The deuterated polyacrylic acid PAA(D) was synthesized in our laboratory [15]. We used two mass ratios (r = [BSA]/[PAA]) r = 5 and r = 20. Sample solutions were made from a freshly stock solution of BSA and PAA that were dissolved in deionized and deuterated water, without adding salt. The pH of the mixture was adjusted by the addition of a strong base (NaOH) or a strong acid (HCl) and measured with accuracy pH-meter (multi-parameter analyzer Consort C862, offering a resolution up to 0.001 pH unit). A pH electrode (SP10T, 3 M KCl, pH ranged from 0 to 14. Temperature compensation: 0 °C to 80 °C) was immersed for 10 h more in 3 M of KCl solution and calibrated at 3 points (pH = 4, pH = 7 and pH = 10). The visual appearance (turbidity) of these complexes changed rapidly with time (3–4 h) to reach a stable state. Measurements of pH, turbidity, zeta potential and hydrodynamic radius performed 24 h after mixing. For some samples, these measurements were repeated after one week to ensure the stability of the complexes.

2.2. Characterization of the samples

2.2.1. Size, zeta potential

In this work, the dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments) was used to determine the particle sizes and the zeta potentials of the protein/polyelectrolyte complexes formed at various ratios. All dynamic light scattering (DLS) experiments were performed at a 173° scattering angle and a wavelength of 514 nm. DLS measurements have been performed in the so-called parallel mode that allowed measuring the correlation function over a wide time range. In this mode the relaxation time τ, which differs by orders of magnitude, could be probed in a single measurement. The measured intensity autocorrelation functions are related to the field autocorrelation functions through the Siegert relation:

$$g^2(t) = A + B\left[g^1(t)\right]^2 \tag{1}$$

where, B is a constant depending on the experimental setup and A is the baseline. τ is related to the apparent diffusion coefficient D by the following relationship:

$$1/\tau = Dq^2 \tag{2}$$

The scattering wave vector q is given by $q = (4\pi n/\lambda)\sin(\theta/2)$, where n is the refractive index, θ the diffusion angle and λ the wave length. We measured the apparent diffusion coefficient D for a large pH range. Further, D can be related to hydrodynamic radius distribution R_h by using the Stokes–Einstein equation, $R_h = k_BT/(6\pi\eta D)$; where k_B, T and η are the Boltzmann constant, the absolute temperature and the solvent viscosity, respectively.

Table 1
Weight average molecular weight Mw, polydispersity I and the polymerization index N for the three PAA used.

	Mw (kDa)	Polydispersity I	Polymerization index N
PAA(5.7 k)	5.7	1.09	79
PAA(57 k)	57.5	1.2	798
PAA(118 k)	118	1.13	1638

2.2.2. Turbidimetry

The transmittances of complexes at different composition and pH were measured, at a wavelength equal to 480 nm, with a spectrophotometer (Thermo Spectronic, Cambridge, UK). At this wavelength, strong signals were obtained from the samples. The trend of the turbidity was the same for the other wavelengths. The apparatus was equipped with a temperature unit (Peltier plate) that provided a good temperature control over an extended time. The transmittance T of the samples could be determined from the following relationship: $T = I_t/I_0$, where I_t is the transmitted light intensity and I_0 is the incident light intensity.

2.2.3. NMR measurements

The NMR spectrometer super-heterodyne with two-way irradiation and a receive path H-NMR experiments were done on an AMX Bruker spectrometer operating at 400 MHz. With this device, we gained a 4 signal to noise ratio resulting in better sensitivity and a gain in time of acquisition. Spectra were recorded after an acquisition of 400 scans with a repeating delay of 4 s.

3. Results and discussion

3.1. The evolution of the hydrodynamic radius of BSA versus pH

We present in Fig. 1 the variation of hydrodynamic radius R_h of the BSA versus pH. One should be reminded that these obtained values, at an angle $\theta = 173°$, are apparent. The obtention of absolute value of R_h requires further study of the second order autocorrelation function $g^2(t)$ as a function of scattering angle θ. Nevertheless, this study provides us with an idea on the evolution of the average size of the protein and its stability. We notice that the hydrodynamic radius R_h of the protein is constant over a wide pH range, $38 < R_h < 44$ Å. These values are close to those mentioned in previous studies [16–19]. The greater increase in the size of the protein at pH < 3 is evidence of the denaturation of the BSA. The change in protein conformation depending on the pH has been intensively studied by scientists, an example of which is the work of Gulam Rabbani et al. [20–22].

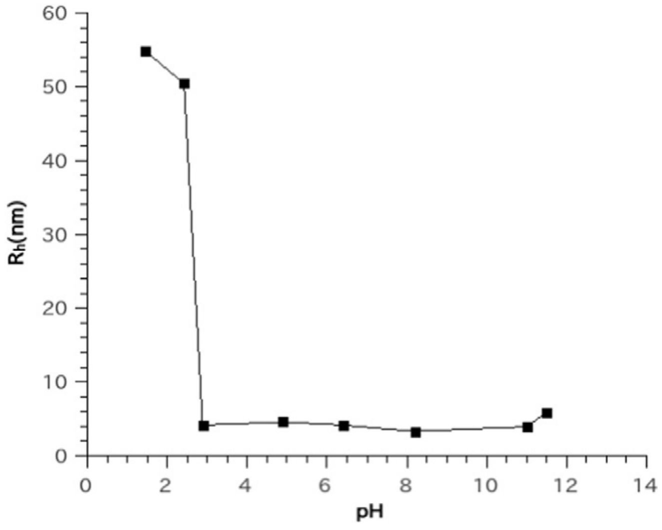

Fig. 1. Evolution of the apparent hydrodynamic radius R_h versus pH. [BSA] = 0.1% (*w*/*v*).